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Effect of gamma radiation in combination with freezing on the microbiological changes in frozen shrimp *Penaeus monodon*

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Abstract

In this study gamma radiation (3, 6 and 9 kGy) in combination with low temperature (-20°C) were applied to retain the quality and shelf-life of shrimp, *Penaeus monodon* for a longer period. The quality was assessed by monitoring microbiological changes (TBC, TMC, TYC, TCC and *Salmonella* count) in irradiated and non-irradiated (control) samples. Among microbiological indicators of spoilage, total bacterial count (TBC) values for irradiated shrimps were found to be 1875, 1625 and 1525 $cfug^{-1}$ of sample at 3, 6 and 9 kGy respectively after 90 days whereas for non-irradiated samples it was found 2475 $cfug^{-1}$ of sample. Total moulds count (TMC) value for non-irradiated samples after 90 days were found 425 $cfug^{-1}$ sample whereas that for irradiated shrimps at 3, 6 and 9 kGy were found to be 275, 250 and 200 $cfug^{-1}$ sample respectively. Total yeast count (TYC) value for non-irradiated samples after 90 days were found 4125 $cfug^{-1}$ sample whereas that for irradiated shrimps at 3, 6 and 9 kGy were found to be 2850, 2150 and 1725 $cfug^{-1}$ sample respectively. Total coliform count and *Salmonella* count showed that those were absent during 90 days storage period. From this study, it was clear that gamma radiation in combination with low temperature showed shelf-life extension (90 days) in each dose of radiation used but during the use of 9 kGy radiation, *Penaeus monodon* showed best quality.

Keywords: *P. monodon,* Gamma radiation, Freezing, Microbiological properties

Introduction

Bangladesh has favorable conditions for shrimp farming. The coastal belt of the country in the south and southeast regions are endowed with favorable tides, salinity and also soil conditions to grow shrimps. Fish is extremely perishable and requires quick preservation. It becomes spoiled by the attack of various pathogenic microorganisms. To extend the shelf life of fish and fishery products, ice storage and rapid chilling, low temperature freezing, modified atmosphere packaging, organic acids, antimicrobials and irradiation techniques are used (Himelbloom *et al.* 1994, Masniyom *et al.* 2002, Al-

Dagal and Bazarra 1999, Gelman *et al.* 2001, Venugopal *et al.* 1999). Shrimps are preserved only by low temperature blocking but the self life of preserved fish is not satisfactory. Various experiments made it clear that no adverse health effects occur when irradiated foods are consumed, using mean doses of irradiation of up to 10 KGy (Rady *et al.* 1988). The validity of this technique is already recognized in many countries, including Canada, USA and the European Union (Tauxe 2003) for many food products including shrimps (Pszczola 1990).

Combination of food irradiation and low temperature refrigeration provide a means to increase shelf life of fish products. In this study effect of gamma radiation (3, 6 and 9 KGy) in combination of refrigeration (-20°C) were applied on shrimp *Penaeus monodon* to observe the increase in shelf life.

Materials and methods

Fish samples

Tiger shrimp used in this experiment were collected from Snow King Frozen Foods (Pvt) Ltd., Mirpur-1, Dhaka and the factory authority collected the tiger shrimp from Rupsa, Khulna. Then blocked (-20°C) tiger shrimp taken in a ice box and immediately brought in the laboratory of Food Processing & Preservation Division, IFRB, AERE, Savar, Dhaka. The entire samples were at first randomly divided into four lots: non-irradiated (control) and irradiated (3, 6 and 9 KGy). Control sample was kept at -20°C within polypropyl polythene bags for preservation purposes without radiation.

Irradiation and storage conditions

Samples were irradiated using a Cobalt^{60} radiation source. Doses applied in this study were 3, 6 and 9 KGy. Before irradiation the samples were kept in polypropylene/polythene bags under atmospheric condition. The non-irradiated and irradiated samples were subsequently stored at -20°C. All samples were examined at the 0, 15, 30, 45, 60, 75 and 90 days of storage period.

Microbiological changes

The total bacterial count (TBC) was determined by decimal dilution technique followed by pour plate technique (Sharf 1966). The microbial changes were estimated by total bacteriological count technique following Withfogel (1962). TBC, TMC, TYC, TCC and *Salmonella* are the valuable measures to assess the degree of freshness of fish.

Results and discussion

Total Bacterial Count (TBC) analysis

TBC values of non-irradiated and 3, 6 and 9 kGy irradiated samples stored at -20°C is shown in Table 1. The initial TBC values were 250, 150, 100 and 100 cfug^{-1} respectively for control sample, 3, 6 and 9 kGy irradiated sample. The TBC values were

2475, 1875, 1625 and 1525 $cfug^{-1}$ respectively for control sample, 3, 6 and 9 kGy irradiated sample at the end of 90 days storage periods. Comparing the treatment, the lowest TBC value was found in the 9 kGy treatment group while the highest value was measured in the control group. The TBC values were gradually increased with the progress of storage period. Under investigation best result of TBC value were found at the treatment dose of 9 kGy.

Table 1. TBC ($cfug^{-1}$) sampled from control and different treatments of doses in irradiated frozen shrimp during 90 days storage period at -20°C. Within column and rows means (±SEM) with different letters denote significant differences (ANOVA HSD, $p<0.05$).

Duration (day)	Level of Radiation (KGy)			
	Control	3	6	9
Control	250 ± 50^{e}	150 ± 50^{e}	100 ± 50^{d}	100 ± 0.00^{e}
15	500 ± 100^{de}	300 ± 50^{de}	175 ± 25^{cd}	225 ± 25^{d}
30	775 ± 75^{cd}	450 ± 50^{cd}	400 ± 50^{bc}	375 ± 75^{cd}
45	1025 ± 125^{bc}	725 ± 25^{bcd}	675 ± 75^{ab}	525 ± 25^{bc}
60	1375 ± 25^{abc}	1025 ± 125^{abc}	925 ± 75^{ab}	925 ± 75^{ab}
75	1800 ± 50^{ab}	1575 ± 75^{ab}	1275 ± 25^{ab}	1275 ± 75^{a}
90	2475 ± 175^{a}	1875 ± 75^{a}	1625 ± 75^{a}	1525 ± 125^{a}

Total bacterial count (TBC) increased with the increase of storage period. Shewan (1975) recommended that TBC 1.0×10^{6} cfu/g of fish flesh is considered as maximum allowable limit. At -20°C storage temperature control, 3 KGy, 6 KGy and 9 KGy irradiated fishes obtained 2475±175 cfu/g, 1875±75 cfu/g, 1625±75 cfu/g and 1525±125 cfu/g of fish muscle at the end of 90 days respectively. According to international commission on the microbiological specification of foods (ICMSF 1982) guideline, acceptable total bacterial count for fish is 10^{6}cfu/g. According to the above mentioned suggestions, irradiated samples remained acceptable up to 90 days of storage period. To detect the days when level of TBC exceed the acceptable limit research should be conducted long time.

Total mould count (TMC) analysis

TBC values of non-irradiated and 3, 6 and 9 kGy irradiated samples stored at -20°C is shown in Table 2. In case of total mould count (TMC), it was found that the population increased with the increase of storage period. At -20°C storage temperature control, 3 KGy, 6 KGy, 9 KGy irradiated fishes obtained 425±25 cfu/g, 275±25 cfu/g, 250±50 cfu/g and 200±0.0 0cfu/g of fish muscle at the end of 90 days respectively. From the present study it was found that the shrimp samples treated by 9 KGy dose contained less mould than all other treated and controlled sample. And with the increase of storage time, the control samples obtained more mould than that of irradiated samples. So, from the total mould count it was found that 9 KGy irradiated fish sample give the best result as preservation method.

Table 2. TMC (cfug^{-1}) sampled from control and different treatments of doses in irradiated frozen shrimp during 90 days storage period at -20^{0}C. Within column and rows means (±SEM) with different letters denote significant differences (ANOVA HSD, p<0.05)

Duration (day)	Level of Radiation (KGy)			
	Control	3	6	9
Control	75 ± 25c	00^{c}	00^{c}	00^{b}
15	100 ± 0.00^{bc}	50 ± 00^{bc}	00^{c}	00^{b}
30	175 ± 25^{abc}	100 ± 50^{ab}	50 ± 00^{b}	00^{b}
45	225 ± 25^{ab}	175 ± 25^{ab}	125 ± 25^{a}	75 ± 25^{a}
60	250 ± 50^{ab}	200 ± 50^{ab}	175 ± 25^{a}	125 ± 25^{a}
75	325 ± 25^{a}	250 ± 50^{ab}	200 ± 50^{a}	175 ± 75^{a}
90	425 ± 25^{a}	275 ± 25^{a}	250 ± 50^{a}	200 ± 0.00^{a}

Total yeast count (TYC) analysis

Total yeast count (TYC) increased with the increase of storage period. Yeast density was shown at Table 3. At -20^{0}C storage temperature control, 3 KGy, 6 KGy and 9 1725 ± 75 cfu/g of fish muscle at the end of 90 days respectively. KGy irradiated fishes obtained 4125±425 cfu/g, 2850±200 cfu/g, 2150±100 cfu/g and 1725±75 cfu/g. From the present study it was found that the frozen shrimp samples treated by 9 KGy dose contained less yeast than all other treated and controlled sample.

Table 3. TYC (cfug^{-1}) sampled from control and different treatments of doses in irradiated frozen shrimp during 90 days storage period at -20^{0}C. Within column and rows means (± SEM) with different letters denote significant differences (ANOVA HSD, p<0.05)

Duration (day)	Level of Radiation (KGy)			
	Control	3	6	9
Control	500 ± 100c	300 ± 100d	150 ± 50d	75 ± 25d
15	625 ± 25bc	475 ± 25cd	350 ± 50cd	175 ± 25cd
30	925 ± 125bc	850 ± 150bc	725 ± 125bc	450 ± 100bc
45	1100 ± 150b	1225 ± 175ab	1125 ± 75ab	825 ± 75ab
60	2500 ± 300a	1650 ± 150ab	1450 ± 150ab	1050 ± 150ab
75	3025 ± 75a	2100 ± 150ab	1825 ± 125a	1475 ± 175a
90	4125 ± 425a	2850 ± 200a	2150 ± 100a	1725 ± 75a

Total coliform count (TCC)analysis

During 90 days of storage period the TCC was absent controlled and all treated sample which is shown in Table 4. According to ICMSF (1986) guideline, acceptable total coliform count for fish is less than 500 cfu/g. So the entire sample was acceptable during whole investigation period.

Table 4. TCC ($cfug^{-1}$) sampled from control and different treatments of doses in irradiated frozen shrimp during 90 days storage period at -20°C

Duration (day)	Level of Radiation (KGy)			
	Control	3	6	9
Control	Absent	Absent	Absent	Absent
15	Absent	Absent	Absent	Absent
30	Absent	Absent	Absent	Absent
45	Absent	Absent	Absent	Absent
60	Absent	Absent	Absent	Absent
75	Absent	Absent	Absent	Absent
90	Absent	Absent	Absent	Absent

Salmonella count analysis

During 90 days of storage period *Salmonella* was absent controlled and all treated sample which is shown in Table 5. According to ICMSF (1986) guideline, acceptable *Salmonella* for fish is absent cfu/25g. So the entire sample was acceptable during whole investigation period. To detect the days of storage when *Salmonella* developed in sample research should be conducted for long time.

Table 5. *Salmonella* ($cfug^{-1}$) sampled from control and different treatments of doses in irradiated frozen shrimp during 90 days storage period at -20°C

Duration (day)	Level of Radiation (KGy)			
	Control	3	6	9
Control	Absent	Absent	Absent	Absent
15	Absent	Absent	Absent	Absent
30	Absent	Absent	Absent	Absent
45	Absent	Absent	Absent	Absent
60	Absent	Absent	Absent	Absent
75	Absent	Absent	Absent	Absent
90	Absent	Absent	Absent	Absent

Shrimp industries in Bangladesh are playing a significant role in the national economy. The present study concludes that irradiation (9KGy) is the best method for long time preservation of fresh fish. To extend the shelf-life of the frozen shrimp, were treated with gamma radiation (3 KGy, 6 KGy and 9 KGy) and stored at low temperature (-20^{0}C) for 90 days for determining the shelf life extension of these fish sample some parameters such as total bacterial count (TBC), total mould count (TMC), total yeast count (TYC), total Coliform count (TCC) and total *Salmonella* count(TSC) were used in every15 days interval. The maximum shelf life was found with radiation dose of 9 KGy.

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